Expedited procedure
Under 37 CFR 1.116 1021.43414X00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: C. UEMATSU, et al.

Serial No: 10/759,179

Filed: JANUARY 20, 2004

Title: METHOD FOR EXPRESSED GENE ANALYSIS AND PROBE KIT FOR EXPRESSED GENE ANALYSIS

Group AU: 1637
Examiner: Stephanie Kane Mummert
Confirm. No: 7828

REQUEST FOR RECONSIDERATION AFTER FINAL REJECTION

Mail Stop: AMEND – NO FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450 April 24, 2007

Sir:

In response to the Office Action mailed January 24, 2007, Applicants respectfully submit the following arguments and explanations traversing the prior art rejections in this Office Action mailed January 24, 2007.

As set forth in the following, Applicants respectfully traverse the rejections of their claims on prior art grounds, in Items 2-4 on pages 2-18 of the Office Action mailed January 24, 2007, and respectfully submit that all of the claims presented for consideration by the Examiner patentably distinguish over the teachings of the references applied by the Examiner in rejecting claims in the Office Action mailed January 24, 2007, that is, the teachings of the U.S. patents to Ovyn, et al., No. 6,110,681, and to Livak, et al., No. 5,538,848, United States Patent Application Publication No. US2001/0039014 to Bass, et al., United States Patent Application Publication No. US 2003/0190646 to Wenz, et al., and the articles by Eun, et al., "Simultaneous Quantitation of Two Orchid Viruses, by the TaqMan® Real-Time RT-

PCR", in Journal of Virological Methods 87 (2000) 151-160; Leone, et al., "Molecular Beacon Probes Combined With Amplification by NASBA Enable Homogeneous, Real-Time Detection of RNA", in Nucleic Acids Research, 1998, Vol. 26, No. 9, pages 2150-2155 (Leone '98), Leone, et al., "Direct Detection of Potato Leafroll Virus In Potato Tubers by Immunocapture and the Isothermal Nucleic Acid Amplification Method NASB", in Journal of Virological Methods 66 (1997) 19-27 (Leone '97), Mackay, et al., "Real-Time PCR in Virology", in Nucleic Acids Research, 2002, Vol. 30, No. 6, pp. 1292-1305, and Rizzo, et al., "Chimeric RNA-DNA Molecular Beacon Assay for Ribonuclease H Activity," in Molecular and Cellular Probes (2002) 16, 227-283, under the provisions of 35 USC 103.

It is respectfully submitted that these references as applied by the Examiner would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, including a step of subjecting the gene to be analyzed to nucleic acid amplification using, inter alia, (a) a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene and including a second base sequence closer to the 5' end of the primer for introduction than the first base sequence, and (b) a probe comprising a base sequence identical or complementary to the first base sequence, together with steps of digesting the probe and detecting fluorescence, and wherein the gene to be analyzed is prepared by the introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, into the target gene so that the second base sequence is bound to a position closer to a 5' end of the gene to be analyzed than the first base sequence. See claim 1.

As will be shown in the following, it is respectfully submitted that none of the references disclose, or would have suggested, either alone or in combination as applied by the Examiner, subjecting the gene to be analyzed to amplification using the primer for introduction including the first base sequence (a target non-specific sequence) at a location between a third base sequence (a target-specific sequence) at the 3' end and a second base sequence (e.g., T7 promoter sequence) at the 5' end, and by introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase into the target gene, in the manner specified in the present claims. Through such introduction and use of the recited nonspecific first base sequence and the probe comprising, inter alia, a base sequence identical or complementary to the first base sequence, an advantage is achieved that the probe used in the present invention does not have to be designed for each use in accordance with the base sequence of the target gene and can be universally used regardless of the target gene. The probe used can amplify and detect any type of target gene under substantially the same conditions, and analysis thereof can be simply conducted. See, e.g., pages 28 and 29 of Applicants' specification.

As will be shown infra, it is respectfully submitted that with the probe described in Wenz, et al., the "AS-SP" portion cannot hybridize with a target, and the probe cannot extend from the "AS-SP" portion, because the "T-SP" portion is located at the 5' end of the probe and the "P-SP" portion is located at the 3' end of the probe. Especially in view thereof, it is respectfully submitted that the probe disclosed in Wenz, et al., including the "T-SP" portion, the "AS-SP" portion and the "P-SP" portion, with the "AS-SP" portion between the "T-SP" and "P-SP" portions, is

functionally different from the primer having the first through third sequences, of the present invention.

In addition, it is respectfully submitted that the teachings of the references as applied by the Examiner would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as in claim 1 as discussed previously, and, additionally, having features as in the dependent claims, including (but not limited) wherein a gene to be analyzed is cDNA including the first and second base sequences introduced therein by introduction with subjecting mRNA of the target gene to reverse transcription using the primer for introduction, as in claim 2; and/or wherein the nucleic acid amplification is conducted by steps as in claims 3 and 4; and/or wherein the nucleic acid amplification is conducted at a substantially single temperature (see claim 5), in particular where such single temperature is between 37°C and 55°C (see claim 6); and/or wherein the RNA polymerase and the second base sequence are as set forth in claim 7; and/or wherein two or more target genes are simultaneously detected in a single reaction vessel using at least two types of probes (see claim 8), in particular wherein such at least two types of probes have substantially the same melting temperature (see claim 9); and/or wherein the probe is a DNA/RNA hybrid strand (see claim 14).

By use of the primer for introduction as in the present claims, which includes the first, second and third base sequences relatively located to the 5' end of the primer, with the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, the first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, together with the probe comprising a base sequence identical or complementary to

the first base sequence, a universal probe for expressed gene analysis which does not have to be designed for each use in accordance with the base sequence of the target gene is achieved. The universal probe according to the present invention can amplify and detect any type of target gene under substantially the same conditions, and analysis thereof can be simply conducted. Note, for example, the second paragraph on page 6 of Applicants' specification; see also the paragraph bridging pages 28 and 29 thereof.

Wenz, et al. discloses a technique for detection of nucleic acid sequences using coupled ligation and amplification reactions. The most general disclosure of the technique described in Wenz, et al. is set forth in paragraph [0007] on page 1 of this patent document. Note also paragraphs [0009] and [0010] on page 1; the Examiner has specifically referred to paragraphs [0028] - [0030] on page 3 of this patent document, and Figs. 1-3 of this patent document.

Wenz, et al. uses a probe including a T-SP portion (a target-specific sequence), a "P-SP" portion (a target non-specific sequence), and an "AS-SP" portion between the "T-SP" portion and the "P-SP" portion. It is respectfully submitted that the "AS-SP" portion cannot hybridize with a target, and the probe cannot extend from the "AS-SP" portion, because the "T-SP" portion is located at the 5' end of the probe and the "P-SP" portion is located at the 3' end of the probe. It is respectfully submitted that Fig. 1 of Wenz, et al. clearly shows this fact, that is, the probe of Wenz, et al. cannot be used as a primer. It is respectfully submitted that Fig. 3 (cont.), on sheet 4 of 7 of the Wenz, et al. publication, shows that an oligonucleotide including the "P-SP", the "AS-SP" and "P-SP" portions in Wenz, et al. is used as a template, in contrast to a primer. As can be seen in the foregoing, it is respectfully submitted that the three portions described in Wenz, et al. are

functionally different from the first, second and third base sequences of the present invention. That is, it is respectfully submitted that the probe described in Wenz, et al. is completely different from the primer for introduction in the present invention, in function as well as in construction.

It is respectfully submitted that Wenz, et al. would have neither taught nor would have suggested various aspects of the present invention, including wherein the primer for introduction includes a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene; or wherein the gene to be analyzed is prepared by the introduction of the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, into the target gene so that the second base sequence is bound to a position closer to the 5' end of the gene to be analyzed than the first base sequence; with use of a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore and at another end with a quencher, as in the present claims, and/or other features of the present claims as discussed previously, and advantages thereof.

Contentions by the Examiner concerning Wenz, et al., in the paragraph bridging pages 19 and 20 of the Office Action mailed January 24, 2007, are noted. However, contrary to the contentions by the Examiner, it is respectfully submitted that the addressable-specific portion of the probe in Wenz, et al. does not serve the same functional role as the first base sequence as in the present claims; and, in particular, as set forth previously, it is respectfully submitted that the addressable-specific portion of the primer in Wenz, et al. cannot hybridize with a target, and the probe cannot extend from the AS-SP portion. Particularly in view of this error by the

Examiner in interpreting the teachings of Wenz, et al., the Examiner is respectfully requested to reconsider the obviousness determination especially with respect to differences between the teachings of Wenz, et al., as well as the other applied references, and the presently claimed invention.

Moreover, it is respectfully emphasized that, as a difference (deficiency) between the teachings of Wenz, et al. and the presently claimed subject matter, Wenz, et al. would have neither taught nor would have suggested such method as in the present claims, including, inter alia, use of the primer for introduction including the first base sequence closer to the 5' end of the probe than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence, as part of the processing as in the present claims, and advantages thereof as discussed previously.

It is respectfully submitted that the additional teachings of the secondary references as applied by the Examiner in Items 2-4 on pages 2-18 of the Office Action mailed January 24, 2007, would not have rectified the deficiencies of Wenz, et al., such that the presently claimed invention as a whole would have been obvious to one of ordinary skill in the art.

Ovyn, et al. discloses oligonucleotides that can be used as primers to amplify a region of the 16S rRNA of *Mycoplasma pneumoniae*. Note column 3, lines 39-45 of this patent. Note especially column 7, lines 5-28, of this patent, describing a method for the detection of the specified microorganism. Note also column 3, lines 39-46; column 5, lines 46-67; and column 6, lines 12-16 and 43-61.

It is respectfully submitted that Ovyn, et al. would have neither taught nor would have suggested, alone or in combination with the teachings of the other applied references, the presently claimed method, including, inter alia, use of the

primer for introduction having the first base sequence as in the present claims, together with the second and third base sequences.

It is respectfully submitted that the upstream and downstream primers in Ovyn, et al. only comprise, respectively, a sequence substantially complementary to the target sequence and a sequence substantially homologous to the target sequence. It is respectfully submitted that this patent would have neither taught nor would have suggested, inter alia, a first base sequence as in the present claims.

Moreover, it is respectfully submitted that the probe described in Ovyn, et al. does not include "a base sequence identical or complementary to the first base sequence". It is respectfully submitted that Ovyn, et al. would have neither taught nor would have suggested such probe including such base sequence identical or complementary to the first base sequence, as in the present claims, and advantages thereof as discussed previously.

Livak, et al. discloses methods of monitoring the process of nucleic acid amplification reactions, especially polymerase chain reactions. Note, in general, column 3, lines 29-47, for the broadest description of this method. See also column 3, lines 48-55.

Eun, et al. discloses simultaneous quantitation of two orchid viruses carried out using the TaqMan® real-time RT-PCR. As for the primer design for the method disclosed in Eun, et al., note Table 1 and the description in Item 2.2 on page 153 of this article.

Even assuming, arguendo, that the teachings of Livak, et al. and of Eun, et al. were properly combinable with the teachings of Wenz, et al., and Ovyn, et al., such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, the base sequences, including

in particular, the first base sequence used together with the second and third base sequence in the primer for introduction, and/or the probe including a base sequence identical or complementary to the first base sequence, and labeled at one end with a fluorophore and at another end with a quencher, and advantages achieved thereby; and/or other features of the present invention as discussed in the foregoing, and advantages thereof.

It is respectfully submitted that the teachings of the references as applied by the Examiner in Items 3 and 4 on pages 9-18 of the Office Action mailed January 24, 2007, would have neither taught nor would have suggested the presently claimed subject matter, including features thereof as discussed previously.

Wenz, et al. has been previously discussed.

Leone '98 discloses employment of molecular beacon probes in a NASBA amplicon detection system to generate a specific fluorescent signal concomitantly with amplification. This article describes the coupling of RNA amplification by NASBA with amplicon detection by molecular beacon technology to produce a homogenous RNA assay, called AmpliDet RNA. Note the first full paragraph in the left-hand column on page 2151 of this article. See also the discussions under the headings "Selection of amplification primers and probe", "Synthesis of the molecular beacons", "NASBA" and "Post-NASBA analysis", on page 2151 of this article.

Leone '97 reports on the development and optimization of the NASBA technology for the direct detection of PLRV (potato Leafroll virus) virions in microfuge tubes and to a post-NASBA analysis amplification products by acridine orange-stained agarose gels. Note the sole full paragraph in the right-hand column on page 20 of Leone '97.

Taking the teachings of Leone '98 even as evidenced by the disclosure of Leone '97 and even in light of the teachings of other references as applied by the Examiner, discussed infra, it is respectfully submitted that the combined teachings of the references would have neither taught nor would have suggested the presently claimed subject matter, including, inter alia, use of the primer for introduction having the first base sequence as in the present claims, and advantages thereof as discussed previously.

Bass, et al. discloses automated devices and systems for performing nucleic acid recombination, mutation, shuffling and other diversity generating reactions in vitro. As applied by the Examiner, this publication discloses that as an alternative to TaqMan® is the use of molecular beacons to assess library quality. Note paragraph [0329] on page 36.

Mackay reports on detection of polymerase chain reaction products during real-time. As applied by the Examiner, note, for example, page 1297, right-hand column, of this article.

Even assuming, arguendo, that the teachings of Wenz, et al., Leone '98 (even as evidenced by Leone '97), Bass, et al. and Mackay, et al. were properly combinable, it is respectfully submitted that such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including use of the primer for introduction having the first base sequence, as in the present claims, and other features of the present invention as discussed previously, including the probe with a base sequence identical or complementary to the first base sequence, and advantages thereof.

In connection with the rejection of claim 14 as set forth in Item 4 on pages 15-18 of the Office Action mailed January 24, 2007, the teachings of Wenz, et al. and Ovyn, et al., have been previously discussed.

The article by Rizzo, et al. discloses preparation of RNA/DNA chimeric molecular beacons, which contain a single-stranded RNA/DNA chimeric oligonucleotide labeled with a 5'-fluorescein as fluorophore and a 3'-DABCYL as quencher, referring to Fig. 1 on page 279 of this article. This article discloses that the fluorophore of the probe is held in proximity to the quencher by the stem-loop structure; and that when the RNA sequence of the RNA:DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested. Note the second full paragraph in the left-hand column on page 278 of this article. Note also the paragraph on pages 279 and 280; and the Conclusions set forth in the left-hand column on page 282.

Even assuming, arguendo, that the teachings of Rizzo, et al., were properly combinable with the teachings of Wenz, et al, and of Ovyn, et al, such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including use of the primer for introduction comprising the first base sequence between the second and third sequences, the first base sequence closer to the 5' end of the primer than the third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence, and wherein the gene to be analyzed is prepared by introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, and with the probe utilized in the method comprising a base sequence identical or complementary to the first base sequence and labeled on one end with a fluorophore and at another end with a quencher.

In view of the foregoing comments, reconsideration and allowance of all claims presently pending in the above-identified application is respectfully requested.

Kindly charge any shortage in fees due in connection with the filing of this paper to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Deposit Account No. 01-2135 (case 1021.43414X00), and credit any excess payment of fees to such Deposit Account.

Respectfully submitted,

ANTONELLI, TERRY, STOUT & KRAUS, LLP

By /William I. Solomon/
William I. Solomon
Registration No. 28,565

WIS/ksh
1300 N. Seventeenth Street
Suite 1800
Arlington, Virginia 22209
Tel: 703-312-6600
Fax: 703-312-6666